EXHIBIT

No. 2

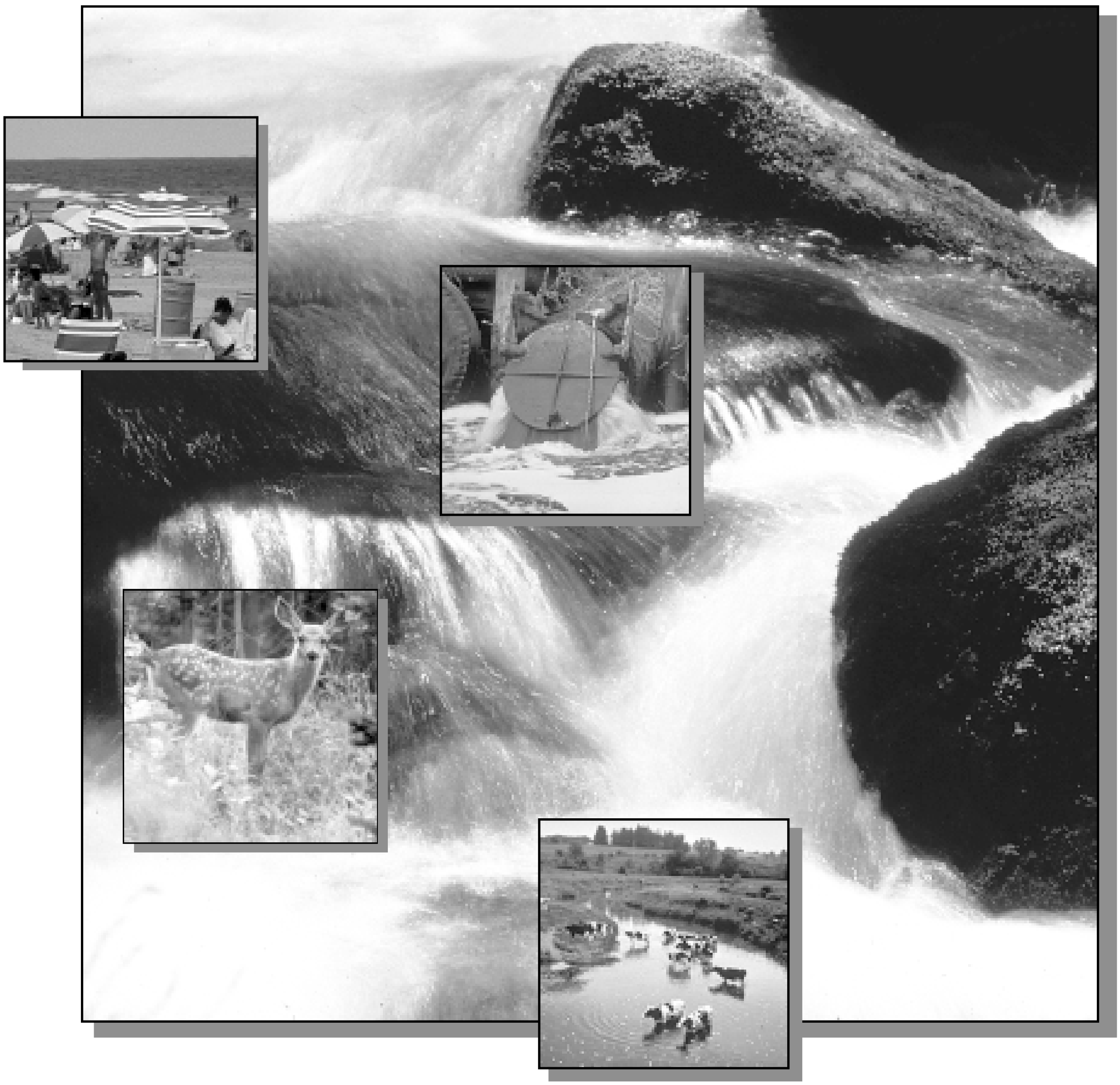
DEFENDANTS' REPLY TO THE STATE OF OKLAHOMA'S RESPONSE TO MOTION TO EXCLUDE THE TESTIMONY OF DR. CHRISTOPHER TEAF
(DOCKET NO. 2156)

05-CV-0329 GKF-PJC

United States Environmental Protection Agency
Office of Water 4503 F Washington DC 20460
EPA 841-R-00-002 January 2001

Protocol for Developing Pathogen TMDLs

First Edition



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Assessment and Watershed Protection Division
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Table 2-3. Viruses of concern to water quality and their associated diseases[1]

Virus	Disease	Effects
Adenovirus (48 serotypes; types 40 and 41 are of primary concern)	Respiratory disease, gastroenteritis	Various effects
Enterovirus (68 types, e.g., polio, echo, encephalitis, conjunctivitis, and Coxsackie viruses)	Gastroenteritis, heart anomolies, meningitis	Various effects
Hepatitis A	Infectious hepatitis	Jaundice, fever
Reovirus	Gastroenteritis	Vomiting, diarrhea
Rotavirus	Gastroenteritis	Vomiting, diarrhea
Calicivirus (e.g., Norwalk-like and Sapporo-like viruses)	Gastroenteritis	Vomiting, diarrhea
Astrovirus	Gastroenteritis	Vomiting, diarrhea

[1] Hepatitis E is an emerging virus that has caused large outbreaks of infectious hepatitis outside of the U.S.

Adapted from Metcalf and Eddy, 1991 and G. Shay Fout, USEPA, 2000

INDICATOR ORGANISMS

The numbers of pathogenic organisms present in polluted waters are generally few and difficult to identify and isolate, as well as highly varied in their characteristic or type. Therefore, scientists and public health officials typically choose to monitor nonpathogenic bacteria that are usually associated with pathogens transmitted by fecal contamination but are more easily sampled and measured. These associated bacteria are called *indicator organisms*. Indicator organisms are assumed to indicate the presence of human pathogenic organisms. When large fecal coliform populations are present in the water, it is assumed that there is a greater likelihood that pathogens are present (McMurray et al., 1998). Fecal indicators are used to develop water quality criteria to support designated uses, such as primary contact recreation and drinking water supply. EPA publishes 304(a) criteria as guidance to states and tribes. States and tribes may adopt EPA's 304(a) criteria, 304(a) criteria modified to reflect site-specific conditions or criteria based on other scientifically-defensible methods. Fecal indicators may also be used to assess the degree of pathogen removal by treatment processes or to detect contamination of distribution systems.

The selection of fecal indicator organisms is a difficult and controversial process. To function as an indicator of fecal contamination in surface water and groundwater, the organism should (1) be easily detected using simple laboratory tests, (2) generally not be present in unpolluted waters, (3) appear in concentrations that can be correlated with the extent of contamination (Thomann and Mueller, 1987), and (4) have a die-off rate that is not faster than the die-off rate for the pathogens of concern. Some commonly used indicators include coliform bacteria and fecal streptococci. Coliform bacteria, which are able to ferment lactose and produce carbon dioxide gas (CO_2), include total coliforms, fecal coliforms, and *Escherichia coli* (*E. coli*). The term "total coliforms" includes several genera of gram-negative, facultative anaerobic, non-spore-forming, rod-shaped bacteria, some of which occur naturally in the intestinal tract of animals and humans, as well as others that occur naturally in soil and in fresh or marine waters and could be pathogenic to a variety of specific hosts. Fecal coliforms (a subset of total coliforms) include several species of coliform bacteria and are found in the intestines and feces of warm-blooded animals. The presence of *E. coli* (a subset of fecal coliforms) in a water sample also indicates fecal contamination since *E. coli* is one of the ubiquitous coliform members of the intestinal microflora of warm-blooded animals (Jawetz et al., 1987). (For more detailed descriptions of these bacteria, see the glossary.) (See Figure 2-1 for indicator organism relationships.)

There has been a resurgence of interest in the enterococcus group as indicators (Davies-Colley et al., 1994). Enterococci (a subgroup of the fecal streptococci [FS] group) are round, coccoid bacteria that live in the intestinal tract. *Streptococcus faecalis* and *Streptococcus faecium* (part of the enterococci family) are thought to be more human-specific than other streptococci, but they can be found in the intestinal tracts of other warm-blooded animals such as cats, dogs, cows, horses, and sheep. The risk to swimmers of contracting gastrointestinal illness seems to be predicted better by enterococci than by fecal coliform bacteria since the die-off rate of fecal coliform bacteria is much greater than the enterococci die-off rate.

the water table, groundwater has traditionally been considered the water source least susceptible to contamination by pathogens. However, depending on soils and geology, connections between groundwater and a contaminated surface or subsurface source can pose threats to the quality of aquifers in the area. Seepage from a waste lagoon, a leaking septic tank, or an improperly designed landfill can result in contamination of aquifer resources.

Wildlife can also be a significant nonpoint source of pathogens in many areas. Many wildlife species are reservoirs of microorganisms that are potentially pathogenic to themselves and to humans. Beaver and deer are large contributors of *Giardia* and *Cryptosporidium*, respectively. Waterfowl such as geese, ducks, and heron also can contaminate surface water with microbial pathogens (Graczyk et al., 1998). These pathogens, such as *Giardia* cysts, are a potentially dangerous health risk for humans, livestock, and wildlife.

Although many nonpoint sources of pathogens are diffuse in nature, some can act as direct sources to a waterbody. Examples of these direct nonpoint sources of pathogens are boat discharges, landfills, waterfowl, and failing septic systems. Boats lacking holding tanks for pumpout contribute human pathogens to surface water; groundwater impacts could occur due to seepage from landfill oxidation ponds that contain fecal bacteria (Metcalf and Eddy, 1991); waterfowl contributions of pathogens are often directly deposited to the waterbody of concern; and failing septic systems may contribute significant pathogen loads directly to a waterbody without significant reduction in numbers, especially in coastal areas or areas of coarse-textured soils or karst geology.

Another potential nonpoint source of pathogens is the resuspension of bacteria indicators and pathogens in sediments. For example, Weiskel et al. (1996) reported significantly increased values of water column fecal coliform density after artificial disturbance of the surface 2 cm of sediments in Buttermilk Bay, Massachusetts. These increased levels of fecal coliform bacteria might indicate the presence of pathogens in the waterbody. The most pronounced increases occurred at sites underlain by fine-grained, high-organic-carbon muds. As runoff during a storm event begins, the discharge and velocity increase, in turn scouring bacteria from the benthic areas of the stream (Yagow and Shanholtz, 1998). This scouring causes increased levels of bacteria concentrations in the water column and decreased levels in the stream sediments. After peak discharge, the bacteria concentrations in the water column decrease at a faster rate than the discharge. This causes the sediment to be deposited downstream, where the sediment bacteria concentrations increase and water column concentrations return to background levels. The increasing usage of recreational waters can cause resuspension of the high numbers of bacterial indicators and pathogens occurring in the sediments (Burton et al., 1987). This creates a potential health hazard from the possible ingestion of the resuspended pathogens.

Although the type of source provides information on the concentrations and possible loads of pathogens to waterbodies, another important consideration is the proximity of the source to the waterbody of concern. Nonpoint sources closer to a waterbody have a greater likelihood to pollute the water than those located farther away, where attenuation factors and dilution will reduce the actual load delivered to the waterbody.

FACTORS INFLUENCING PATHOGEN SURVIVAL

Determining what happens to the microorganisms once they reach the waterbody is often as challenging as identifying and tracking their sources. As living organisms they require certain conditions to survive, grow, and reproduce. Thus, risks to human health can be increased or decreased depending on water temperature and other factors associated with the waterbody. Many factors influence the die-off rate of viruses, bacteria, and protozoans in the environment. These factors include sunlight, temperature, moisture conditions, salinity, soil conditions, waterbody conditions, settling, association with particles, and encystation. Many other factors affect the die-off rate of pathogens, but not all are described in this protocol. Some of these other factors include the age of the fecal deposit, pH, starvation, structural damage, chemical damage, predation (Davies-Colley et al., 1994), osmotic stress in moving from fresh to marine waters, nutrient deficiencies, turbidity (water clarity), variation of spectral quality of sunlight, microbial composition of effluents, and oxygen concentrations. Some of these factors have a direct influence on mortality, whereas others indirectly affect die-off in the environment by